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DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L10	('5922591')[ABPN1,NRPN,PN,WKU]	3	L10
L9	L8 and hydrophilic	19	L9
L8	L7 and parallel	79	L8
L7	L6 and (mov$ or transport$)	104	L7
L6	L5 and ((multiple$ near5 sample$) or (plurality near5 sample$1))	171	L6
L5	L4 and polymerase chain reaction$	748	L5
L4	different near5 temperature$1	66699	L4
L3	l1 and DNA	0	L3
L2	L1 and (chemic$ or biolog$)	0	L2
L1	metal near5 bar near5 plurality near5 temperature$1	1	L1

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L9: Entry 4 of 19 File: USPT Apr 23, 2002

DOCUMENT-IDENTIFIER: US 6375817 B1
TITLE: Apparatus and methods for sample analysis

Brief Summary Paragraph Right (4):
Recently, efforts have been made to streamline chemical processes to reduce costs, increase accuracy, and improve reaction yields. For example, capillary electrophoresis techniques have been proposed to increase resolution in immunoassays. Various attempts have been made to enhance other common analytical techniques, such as the polymerase chain reaction (PCR). For example, U.S. Pat. No. 5,273,907 reports a capillary pre-loaded with PCR reagents which is used to deliver a sample to the reagents for DNA amplification. Similarly, International Patent Publication WO 93/22058 describes a micro-scale device for performing PCR In this case, PCR reagents from a first chamber are mixed with sample in a second chamber by movement of materials through channels in a microchip.

Brief Summary Paragraph Right (5):
Recently, efforts have been made to streamline chemical processes to reduce labor and complexity. One such effort involves the use of microchip assemblies. A microchip assembly typically consists of a thin silica substrate or other polymeric substrate onto which channels are etched. The channels serve as means for reagent transport and/or as the reaction chambers themselves. Microchip assemblies for performing micro-scale chemical reactions may comprise a series of interconnected channels. For example, channels may be etched onto the surface of a microfabricated solid. Reagents in solution then are placed into the channels, and allowed to react with, e.g., reagents already in the channels. Voltage gradients may be used to control sample flow and mixing. See, e.g., International Publication WO 96/04547. Hydrogen and oxygen gas often results from use of voltage gradients to control sample flow. Electrolysis products also may accumulate near the electrode surface.

Brief Summary Paragraph Right (8):
Apparatus and methods have been developed for rapid, automated analysis of microscale samples using pressure differentials. A sample plug formation device of the invention generally comprises two intersecting channels, an introduction channel and a separation channel. A sample is introduced through an opening in a first channel, referred to herein as a sample introduction channel. The sample moves through the sample introduction channel by vacuum, pressure, capillary action, or a combination thereof. At a distance from the point of sample introduction, the sample introduction channel forms a juncture or junction with (i.e., intersects) a second channel, referred to herein as a separation channel. Through the use of pressure and/or vacuum applied to the separation channel and/or the sample introduction channel, a portion of the sample is transported into the separation channel as the bulk sample crosses the junction between the sample introduction and separation channels. With the proper control, a discrete plug of sample reproducibly may be formed in the separation channel and subjected to separation techniques and/or analysis. Subsequent to the formation of the sample plug, the portion of sample which does not form the sample plug typically is moved to a waste outlet.

Brief Summary Paragraph Right (9):
Formation of the sample plug at the channel junction is controlled by application of pressure differentials in and between the sample introduction and separation channels. A first pressure differential is applied so as to induce sample flow through the introduction channel to the juncture. Subsequently, at least a second pressure differential is applied to move a portion of the sample into and along the axis of the separation channel. A plug of sample generally is formed in the separation channel at the junction when pressure is increased axially along the separation channel relative to the sample introduction channel. The frequency and size of plug formation is

controlled by controlling the pressure differentials.

Brief Summary Paragraph Right (14):
A preferred sample delivery device has a housing defining a capillary which has an open end for introduction of a sample and a closed end. The closed end preferably is associated with a temperature control device which is used to control movement of sample and reactants into the sample delivery system. Immobilized within the capillary may be chemical reagents, such as, binding proteins, ligands, receptors, antibodies, or antigens. These reagents may be detectably labeled and, preferably, are fluorescently labeled. These reagents also may be chemically or enzymatically labelled, e.g., to permit amplification prior to detection.

Drawing Description Paragraph Right (2):
FIG. 1 is a schematic diagram of a sample plug formation device of the invention. A sample introduction channel forms a non-parallel juncture with a separation channel in which a sample plug is formed. The direction of sample flow is shown by arrows 16, 18, and 20.

Detailed Description Paragraph Right (10):
Methods of the invention provide for the formation of a sample plug and, in certain embodiments, for separation of the components of that sample plug. The application of pressure/vacuum serves to push or pull the sample through the channels of the injection system of an apparatus of the invention. After a sample plug is formed in the separation channel, its components may be separated, preferably by electrophoresis. However, a metered sample plug may be transported to another analytical device for analysis without further separation. FIGS. 2A-2F illustrate the various stages of sample plug formation. Numerous means exist for introducing a sample into the apparatus, e.g., injection from a syringe or the like, or introduction from a sample delivery system known in the art. In another embodiment, an absorbent material, such as cotton, may be placed in the introduction channel. The absorbent material attracts sample into the sample introduction channel by capillary action. In another embodiment, an absorbent material may be placed in contact with the outlet of the sample introduction channel, e.g., above the channel, so that the absorbent material draws the sample through the sample introduction channel by creating a vacuum from surface tension (capillary action).

Detailed Description Paragraph Right (12):
Application of a pressure differential to the separation channel 12 for a specific interval reproducibly produces discrete sample plugs. In FIG. 2D, application of positive pressure to the separation channel from the direction opposite of sample 22 flow causes a plug of sample 14 to form downstream of the junction 11 while the remaining sample is moved to waste or back to the introduction location. In certain embodiments, the positive pressure may continue to move the sample plug through the separation channel to effect separation similar to operation of a chromatography column.

Detailed Description Paragraph Right (13):
In a preferred embodiment, the separation channel contains a sieving medium for separation of the sample components based on charge or size. The sieving medium may comprise, for example, polyacrylamide, agarose, polyethylene oxide, polyvinyl pyrolidine, and methylcellulose. Other sieving media such as chromatography particles may be used depending on the particular application. In FIG. 2E, the components of the sample are moved electrophoretically along the separation channel and are separated by application of a voltage gradient along the longitudinal axis of the separation channel 12. Separation of the sample components is achieved by standard electrophoretic methods. Finally, subsequent to separation and/or analysis, FIG. 2F depicts positive pressure being applied from both directions along the separation channel to force the sample remnants out of the channels to cleanse the system.

Detailed Description Paragraph Right (17):
Referring to FIGS. 3A-3F, the method of sample plug formation referred to as "stacking" is depicted using an apparatus of the invention. FIGS. 3A-3F show a series of diagrams similar to those in FIGS. 2A-2F, except that the ionic strength of the media in the sample introduction channel 10 is lower than the ionic strength of the medium in the separation channel 12. In addition, a voltage gradient is applied to the

separation channel 12 while a vacuum moves samplealong the sample introduction channel 10 as shown in FIG. 3B. As understood by a skilled artisan, ionic species moving in an applied electric potential from a lower ionic strength medium to a higher ionic strength medium will experience a decrease in their rate of movement due to a decreased electric field in the higher ionic strength medium. Accordingly, as shown in FIG. 3B, the ionic species "pile up" or concentrate at the interface 24 of the two differing ionic strength media. With the continuous flow of sample through the juncture, the ionic species in a sample may become highly concentrated at the interface 24. Subsequent to the appropriate amount of "stacking," the electric potential is removed and a pressure differential applied to the separation channel to form a sample plug which is concentrated in the ionic species of the sample as shown in FIGS. 3C and 3D. The remaining steps of the method, FIGS. 3E and 3F are as described above for FIGS. 2E and 2F.

Detailed Description Paragraph Right (18):
The procedure of stacking may be useful to concentrate components in dilute samples so a detectable amount of the component or components of interest are transported through the separation channel. Alternatively, a sample medium may be diluted using "anti-stacking." Essentially the same procedure is practiced, however, the sample is in a higher ionic strength medium than is present in the separation channel. Accordingly, an optimized sample plug, e.g., its size and concentration of components, may be controlled by the above techniques, i.e., stacking, anti-stacking and non-stacking.

Detailed Description Paragraph Right (19):
FIG. 4 illustrates a preferred embodiment of the invention. A microchip assembly 25 etched with a series of sample introduction channels 10 and separation channels 12 is shown. The channels meet to form a junction 11. As depicted, the microchip has a plurality of sample introduction and separation channels. The channels are between about 10 .mu.m and about 100 .mu.m in width and about 0.1 .mu.m to about 1000 .mu.m in depth. The microchip also has manifolds 26 and 27 for transporting a sample and/or reagents to and/or within the separation and sample introduction channels.

Detailed Description Paragraph Right (26):
A sample delivery system as described above may be positioned over the opening of a sample introduction channel of a microchip assembly 25 for delivery of a sample for analysis. In operation, the sample delivery systems pick up sample, allow the sample to react with the chemical reagents immobilized on their walls and then deliver the products of the reaction, if any, to the sample introduction channels of a device of the invention. As shown in FIG. 5, an array of such sample delivery systems simultaneously can deliver a plurality of samples to a plurality of sample introduction channels on a microchip assembly 25.

Detailed Description Paragraph Right (27):
Modulation of temperature near the closed end of the capillaries by the temperature control device 32 controls the pick up and delivery of samples, i.e., the movement of sample within the capillaries. Since the capillaries are sealed at one end, heating and cooling the gas in the closed end of the capillaries causes that gas to expand or contract, respectively. When the gas is heated, the volume it occupies, and hence the pressure in the capillary, increases approximately according to the perfect gas law PV=nRT, where P is pressure, V is volume, n is the number of gas molecules, R is the constant 8.314 JK.sup.-1 mol.sup.-1, and T is the temperature. Gas is therefore forced through the opening in the capillary when the gas is heated. The capillary then is submerged in the sample and cooled. Upon cooling, the gas in the capillary contracts and the sample enters the capillary. As the gas in the capillary contracts, the pressure in the capillary decreases. The pressure differential between the outside and inside of the capillary forces sample into the capillary.

Detailed Description Paragraph Right (37):
In operation, the sample suspected of containing a target polynucleotide sequence is brought into contact with the first set of reagents in the capillaries (i.e., the PCR reagents) by heating the closed end of the capillary to expel gas, placing the capillary in the sample, and then cooling the closed end of the capillary to contract the gas and thus draw in the sample. Once the capillary is sufficiently cooled, the sample contacts the first set of chemical reagents. The second temperature control

device 42, placed at a position on the reactor occupied by the PCR reagents, controls thermocycling. The second temperature control device 42 first increases temperature at the position occupied by PCR reagents in order to denature double-stranded DNA in the sample. The same, or a different, temperature control device then cools the reactor region containing the PCR reagents to cause annealing of PCR primers to single-stranded template strands. Heating to a temperature intermediate between that for denaturation and that for annealing causes primer extension. A number of such cycles are repeated until the reaction is complete. The number of PCR cycles, as well as the precise reagents used vary depending upon the amount of available template DNA, reaction efficiency, and other known factors. General protocols and parameters for PCR are known, and are available, for example, in Short Protocols in Molecular Biology, 15-1-15-40 (Ausebel, et al., eds. 1995), incorporated by reference herein.

Detailed Description Paragraph Right (38):
Once PCR is complete, the amplified target sequence is brought into contact with the set of complementary probes by cooling the gas in the closed end of the capillary with the temperature control device 32. The skilled artisan recognizes that a single temperature control device may be used to heat or cool the capillary in order to move sample, and to heat or cool discrete capillary regions for PCR. However, as shown in FIG. 6, separate temperature control devices are preferred. The set of probes may comprise multiple copies of a single probe that is known to hybridize with at least a portion of the amplified target, or the set may comprise a plurality of different probes, some hybridizing to portions of the target, some being non complementary to the target.

Detailed Description Paragraph Right (39):
The sample, comprising amplified nucleic acid, is brought into contact with the probes by cooling the capillary in order to cause sample to move up (away from the point of gas/sample entry) and into contact with the region of the capillary comprising probes, as referred to above. Sample is allowed to incubate with the set of probes for a time sufficient to cause hybridization to a desired level of stringency. The hybridization parameters (i.e., time, buffer, salt concentration, temperature, etc.) are determined based upon sequence length, G/C content, desired stringency, and other criteria known to the skilled artisan. See, e.g., Ausubel, supra at 6-7. Once a desired level of hybridization has been achieved, sample (including hybrid duplex formed between target and probe) is eluted into a sample plug formation device of the invention by further heating the reactor. Eluted sample may then be washed to remove excess (unbound) label. The target polynucleotide sequence is then detected using a sample analysis apparatus of the invention to determine the presence and/or amount of target DNA.

Detailed Description Paragraph Right (44):
After drying of the sample to the card, the card membranes are steam heated using sterile deionized water from unit 58 so as to extract the sample components into a small quantity of liquid. The capillaries of the reactors 48 are then heated to expel gas, moved into position over the membranes, and dipped into the liquid containing the sample. Upon cooling of the capillaries, the gas in the closed end of the capillaries contracts and sample is drawn into the capillaries. The capillaries are preferably pre-loaded with the reagents specific for the immunoassay or polynucleotide detection to be performed, as described above.

Detailed Description Paragraph Right (45):
The sample once reacted in the capillaries, as described above, is deposited in the sample introduction channels of the microchip assembly 25. The capillaries move over so that they are positioned above the sample introduction channels. The capillaries are then heated by temperature control device 32 so that gas inside the closed end of the capillary expands and forces sample out of the capillary, as described above with respect to FIG. 5 and FIG. 6. Once used, the sample delivery systems can be disposed of and new systems containing reagents for the next reaction of interest can be inserted into the integrated device.

Detailed Description Paragraph Right (52):
In addition, since the channels typically are not used directly in an analytical technique, the channels may be readily moveable and have a higher degree of tolerance for positioning with a sample plug formation device of this invention. That is, since the detection system of an analytical device typically remains stationary, the optical

alignment of a liquid detection capillary needs to be done once for optimal accuracy during the analysis of a plurality of samples. Furthermore, if the sample delivery system contains a chemical reagent and is used to perform a reaction, any particulates present or formed during the reaction easily can be filtered prior to introduction of the reaction products to a sample plug formation device thereby preventing clogging and/or inaccurate analysis. These above features permit simple and inexpensive automation robotics to be used.

Detailed Description Paragraph Right (53):
Compared to using traditional systems which rely on capillary action to deliver, mix and/or react chemicals, a sample delivery system of the invention exhibits several advantages. The surface of a channel of a sample delivery system of the invention may be hydrophilic or hydrophobic in contrast to a capillary action surface which requires a hydrophilic surface. Also with respect to the surface of the channel, the reproducibility of sample solution metering is less dependent of the surface characteristics and sample constituents. In addition, the sample delivery system of the invention allows direct control over the metering of samples and reagents, and permits bubble segregation to be practiced routinely. These benefits not only are achieved by the sample delivery system described above, but also with respect to a sample plug formation device of the invention which uses pressure differentials.

CLAIMS:

1. A sample plug formation device comprising:

a housing defining

a separation channel comprising a longitudinal axis, and

an introduction channel which forms a juncture with the separation channel;

a pressure control device independently in communication with the separation channel and the introduction channel wherein

a first pressure differential applied to the introduction channel transports a sample to the juncture, and

a second pressure differential applied to the separation channel transports a portion of the sample in the juncture into the separation channel to form a sample plug; and

a voltage generator in communication with the separation channel, wherein the voltage generator is adapted to apply an electric potential along the longitudinal axis.

8. A method for forming a sample plug comprising the steps of:

(a) providing a sample plug formation device comprising:

a housing defining

a separation channel comprising a longitudinal axis, and

an introduction channel which forms a juncture with the separation channel; and

a pressure control device in communication with the separation channel;

(b) applying a first pressure differential to the introduction channel to transport a sample in communication with the introduction channel to the juncture;

(c) applying a second pressure differential to the separation channel to transport a portion of the sample in the junction into the separation channel to form a sample plug; and

(d) applying an electric potential along the longitudinal axis of the separation channel.

12. The method of claim 8 further comprising the step of applying positive pressure to the separation channel to move the sample plug along the separation channel.

16. A scientific instrument comprising:

a microfabricated solid comprising,

a housing defining

a separation channel comprising a longitudinal axis,

an introduction channel which forms a juncture with the separation channel, and

a pressure control device independently in communication with the separation channel and the introduction channel wherein

a first pressure differential applied to the introduction channel transports a sample to the juncture, and

a second pressure differential applied to the separation channel transports a portion of the sample in the juncture into the separation channel to form a sample plug;

a voltage generator in communication with the separation channel to apply an electric potential along the longitudinal axis;

a computer in communication with the pressure control device to control the pressure control device; and

a detector spaced apart from the junction and in communication with the separation channel to detect a chemical component.